Circulating Biomarkers of Tumor Invasiveness for Diagnosis and Prognosis of Cancer Metastasis

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Abstract: Cancer is one of the serious sapping health problems that threatens humans due to its incurable nature. The only hope of beating cancer is early detection that could provide considerable management and accepted prognosis. Metastasis is the dissemination of the tumor cells from the affected organ to another which is not directly connected to the primary affected site. Once metastasis is predictable, the prognosis is poor, and mortality rate is high. Hence, early diagnosis of pre-metastatic malignant tumors could improve treatment efficacy and hence patients’ survival. The past three decades had witnessed an enormous progress in the understanding of the pathophysiological basis of cancer. Disturbed metastatic biomarkers can predict the possibility of future development of metastases, disease progression and therapeutic efficacy. This review collects some of the most important biomarkers that were associated with metastasis in various types of cancer; MRP1, MMP-9, TGF-β, Vimentin, Snail-1 and TWF and E-cadherin. These biomarkers proved their ability to predict who is most at risk of developing metastasis. MRP1, MMP-9, TGF-β, Vimentin, Snail-1 and TWF has been reported to be upregulated during metastasis whereas E-cadherin has been reported to be downregulated. The close monitor of these biomarkers’ levels could Provide oncologists better understanding of the disease stage and hence help them taking right decisions and begin individualized treatment strategies.

Keywords: Cancer, biomarker, metastasis, diagnosis, prognosis, E-cadherin, MRP1, MMP9, Vimentin, TGFβ, Snail-1, Twinfilin.

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1. INTRODUCTION

Cancer is a major public health problem worldwide and the second leading cause of death in the world. In Egypt, age-standardized incidence rates from that registry are 96.5 and 132.6/100,000 males and 97.3 and 122.1/100,000 females. The commonest sites of cancer in males are liver (18.7%), bladder (12.7%), non-Hodgkin's lymphoma (11.0%) and trachea, bronchus, and lung (8.2%). The commonest sites in females are breast (38.8%), non-Hodgkin's lymphoma (8.5%), liver (4.6%), and ovary (4.5%)1.

Despite the considerable advancement in new-developed therapies for cancer, the overall mortality and morbidity for cancer are high and the prognosis of patients remains disappointed. On the one hand, it might be due to that the time of diagnosing cancer is always at the advanced stage; on the other hand, clinicopathological features of cancer, such as tumor invasion, and metastasis influence the prognosis of patients with cancer. Consequently, new biomarkers that could be used effectively to anticipate the prognosis of patients with cancer are in urgent need2.

In this research, we aim to review the current state of knowledge on metastasis and circulating biomarkers of tumor invasiveness and their potential clinical applications for the diagnosis and prognosis of cancer metastasis by summarizing the importance of some of the most recently discovered metastatic biomarkers, focusing on seven of them. This study was conducted in the period between May 2022 and March 2023. The terms "Metastatic Biomarkers, diagnosis, prognosis, and the selected individual markers as MRP1, etc." were used to locate research articles and reviews published in English over the preceding ten years. ScienceDirect and PubMed were...
among the online medical resources searched. The primary papers and reviews received the most attention.

2. METASTASIS

2.1 Definition of Metastasis

Metastasis is the growth of a secondary tumor in an area of the body that is far from the original cancer site. Tumor cells must depart their originating site, travel through the bloodstream, exert pressure on blood arteries and travel to a secondary site (Figure 2).

2.2 Steps of Metastasis:

2.2.1 Dissemination of cancer cells:

As a result of continuous mistakes in chromosomes segregation during mitosis, chromosomal instability arises. Micronuclei burst due to faults in chromosomal segregation releasing genomic DNA into the cytosol, which then triggers nuclear factor kappa B (NF-κB) signaling and cytosolic DNA-sensing pathways.

2.2.2 Local invasion:

Cancer metastasis starts firstly by local tissue invasion around the initial tumor and degradation of the basement membrane and extracellular matrix (ECM). This step is achieved by boosting the Epithelial-mesenchymal transition (EMT) process.

The predominant mechanism affect cancer metastasis is epithelial-mesenchymal transition. Epithelial cells are immotile and strongly adhere to each other and to the ECM, whereas a mesenchymal cells are more vulnerable to invasion and metastasis.

2.2.3 Intravasation

The infiltration of cancer cells into a blood vessel or lymphatic channel through the basement membrane (BM) is the definition of intravasation. The tissues' structural limitations apply some mechanical pressure to the invasive tumor cells during intravasation. Genomic rearrangement is a result of nuclear compression and raises the risk of metastatic spread.

2.2.4 Circulation

Most circulating tumor cells (CTCs) circulate alone; however, some cells travel in groups with the aid of neutrophils. Additionally, Neutrophils inhibit the activation of leukocytes which improves the possibility of survival of CTCs. Once CTCs are in the capillaries, they undergo one of the two routes: extravasation or proliferation.

2.2.5 Extravasation

Circulating tumor cells get stuck when they go through tiny capillaries. This either triggers extravasation of the cell or microvascular rupture. The extravasation of cancer cells is facilitated by the release of cytokines into the bloodstream and the activity of platelets.

2.2.6 Outgrowth (colonization)

Usually a fraction of about (0.01%) of the whole cancer cells survive and develop additional tumors. Both dormancy and proliferation are options for the cells that survive. Typically, tumor cells infiltrate secondary sites and go into dormancy for a while.

Figure 1. Epithelial-mesenchymal transition Cascade (Self constructed and designed by authors using biorender).
2.3 Metastatic biomarkers

The role of metastatic biomarkers whose expression levels change dynamically in tumor and have much association with tumor invasion and metastasis, has attached more attention. These molecules could serve as potential markers providing a prognostic significance for patients with cancer 15.

2.3.1 E-cadherin

E-cadherin (E-CAD, Cadherin1, Cdh1) is a transmembrane protein and the main building block of adherent junctions connecting epithelial tissues. It is required for integrity of tissue, junctional maintenance and dynamics 16.

Cadherin expression is regulated by progesterone and endometrial calcitonin, which has an effect on the compactness of uterine tissue and embryo implantation 17.

Adherens junctions (AJs) are signaling hubs created when the extracellular domains of cadherins connect with their cytoplasmic tails. Cadherins on nearby cells interact with one another through AJs to create a zipper like structure. E-cadherin through its cytoplasmic domain interacts with p120 catenin and beta-catenin to create complex that links AJs to the actin cytoskeleton 18. Later, cadherins restrict cancer cell movement and progression in mature tissues by maintaining cellular polarity, tissue integrity, and homeostasis 19.

2.3.2 Motility related protein-1

Motility related protein-1 (TSPAN-29), Cluster of differentiation (CD9)) is one of the key tetraspanin family members, which orchestrate lateral interactions with other membrane proteins like integrins 20.

Motility related protein-1 has several biological activities like motility, cell migration, proliferation, fertilization, tumor metastasis and differentiation 21. It participates in many functions, such as extracellular vesicle formation and the subsequent contribution of those structures to cell signaling 22. Besides its physiological functions, MRP1 was found to have a major role in tumor biology field, where abnormal level causes neoplasia and metastasis 23.

2.3.3 Matrix Metalloproteinase-9 (MMP-9)

Matrix Metalloproteinase-9 (MMP-9) is a protein with endopeptidase activity. Extracellular matrix (ECM) and BM attachment are the main substrates for MMP-9. It has three repeats of fibronectin which can attach to and digest collagen which is one of the crucial elements of the basement membrane 24.

It is implicated in numerous biological processes as a result of its extracellular environment proteolytic cleavage activity. Among these biological processes are proteolytic breakdown of ECM, modification of the connections between cells and the ECM, cleavage of proteins on cell surfaces and in the extracellular environment.

2.3.4 Vimentin

Vimentin is a cytoskeletal filament protein in addition to other elements, such as microfilaments and microtubules 25. Vimentin is widely considered as a biomarker of EMT since its level increases during tumor metastasis 26.
Vimentin has a significant role in several physiological processes and is crucial for regulating cell function. Vimentin provides anchoring effect in the organelle and keeps the normal morphology of cells. Vimentin also takes part in cell migration, proliferation, metastasis, and invasion at the cellular level. It contributes to the growth of mammary glands, neurological system, and angiogenesis concerning function of organ. Vimentin can also regulate pathological processes like growth, wound healing and metastasis, according to many studies. In cells with high mobility, vimentin was shown to be highly expressed 27.

2.3.5 Transforming growth factor beta (TGF-β)

Transforming growth factor beta (TGF-β) is a group of released cytokines that have three different isoforms: TGFβ-1, TGFβ-2, and TGFβ-3. Each one of the TGF-β family isoforms has a unique function and expression pattern 28. It has been hypothesized that the TGFβ-1 isoform is crucial for cancer aggressiveness 29. It has many physiological functions including wound healing, inflammation, differentiation, and embryogenesis. Additionally, it takes part in the pathological processes like diabetes, cancer, and neurological disorders 30. TGF-β may stimulate tumor proliferation and metastasis in later stages of cancer.

2.3.6 Snail-1

Snail-1 is a zinc-finger transcription factor 31. Snail-1 is a protein having 264 amino acids. The 2.0 kb, 3-exon SNAI1 gene, which is found on chromosome 20q13.2, encodes the Snail-1 protein in humans. Human kidney, thyroid, lungs, lymph nodes, liver, placenta and skeletal muscle tissues physiologically express Snail-1 32,33.

Physiologically, Snail-1 contributes to cell survival, wound healing, and embryo implantation 34. The Snail superfamily is essential for many processes that are connected to apoptosis, metastasis, differentiation and drug resistance 35. Snail-1 controls important genes involved in carcinogenesis 36. It has received substantial research as a crucial EMT marker 37. Furthermore, it is vital to fibroblast activation 36, which is important to cancer-associated fibroblasts' growth and controls tumor metastasis or immune system evasion 38.

2.3.7 Twinfilin

Twinfilin is actin binding protein. It attaches to ADP-G actin to stop G actin from binding to actin filaments 42. TWF-1 and TWF-2 are two isoforms found in mammals that have differing subcellular locations and tissue distributions 43. Twinfilin participates in many developmental processes, like platelet activation, cell migration, endocytosis, cell motility, and axonal growth of neurons 44. It also controls the dynamics of cytoskeletal structure that involves interactions with actin filaments, actin monomers and is implicated in sensitivity to drugs. Furthermore, TWF is vital in regulating EMT in cancer and chemoresistance. TWF also functions as regulator of metastasis and cell cycle 45.

Table (1): The key genes involved in malignancy regulated by Snail-1

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Common function</th>
<th>Role of Snail-1</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cadherin</td>
<td>Cell-cell adhesion</td>
<td>Gene suppression</td>
<td>(39)</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Cytoskeleton building, Mesenchymal marker</td>
<td>Gene upregulation</td>
<td>(40)</td>
</tr>
<tr>
<td>MMP</td>
<td>ECM degradation</td>
<td>Gene upregulation</td>
<td>(41)</td>
</tr>
</tbody>
</table>

3. Diagnostic significance of metastatic biomarkers:

3.1 E-cadherin in cancer metastasis

Cell-cell adhesion is critically dependent on E-cadherin. Without E-cadherin, cells start to grow on top of each other initiating cancer 46. Hence, E-cadherin might prevent epithelial cells from initially separating from the original mass of tumor. When cell junctions and cell-cell adhesion are lost, this will enable cells to move to distant places and invade adjacent tissues 47. Additionally, the activity state of E-cadherin at the cell surface controls the cancer metastatic process (Dissemination of cancer cells, invasion, intravasation, circulation, extravasation and colonization) 48. Therefore, cadherins are frequently inactivated or functionally inhibited.
during carcinogenesis enabling the metastatic processes to occur \(^{20}\).

Canel and his colleagues \(^{40}\) stated that in vitro invasion and dissemination were found to increase when E-cadherin was lost demonstrating that E-cadherin inhibits cell invasion and acts as a metastasis suppressor.

Sinkevicius et al. \(^{50}\) described that in lung adenocarcinoma models, loss of E-cadherin promoted tumor growth and exacerbated metastasis to lymph node or chest wall. In addition, Siegel et al. \(^{51}\) observed in lung cancer patients with low survival rates that E-cadherin loss was associated with a high incidence of brain metastases.

Moreover, Christou et al. \(^{52}\) found that E-cadherin loss promoted colorectal carcinoma cell (CRC) metastasis. Drury et al. \(^{53}\) stated that tumor stage, invasion depth, and lymph node metastasis are associated with downregulated expression of E-cadherin in CRC patient tumors. Moreover, reduced E-Cadherin level has been shown in lobular breast cancer metastasis \(^{54}\), diffuse gastric carcinoma \(^{55}\), and pseudopapillary neoplasm of the pancreas \(^{56}\).

Epithelial mesenchymal transition (EMT) has been proposed to have a strong link with aggressiveness of tumor and metastatic proclivity \(^{57}\). A meta-analysis included 1939 individuals with Head and Neck Squamous Cell Carcinoma revealed that downregulation of E-cadherin was predictive for poor survival and a considerably greater likelihood of metastatic development \(^{58}\). In another report by Venhuizen et al. \(^{59}\) stated that loss of E-cadherin was thought to be a fundamental characteristic of invasive lobular carcinoma, with 87% of invasive lobular carcinoma and just 7% of non-lobular breast cancers were E-cadherin-negative.

3.2 Motility related protein -1 in cancer metastasis

Previous research work had reported the presence of a connection between MRP1 and cancer cell proliferation, survival, metastasis and prognosis \(^{60}\). Studies by Baek et al.\(^{61}\) and Liang et al. \(^{62}\) reported in acute lymphoblastic leukemia and breast cancer patients, a strong relation between increased MRP1 level and worse prognosis. Motility Related Protein-1 was proposed as a biomarker candidate for metastatic cell renal cell carcinoma (RCC), which might be used to foretell RCC's propensity for metastasis as well as to discriminate between different cancer subtypes \(^{63}\). Moreover, MRP1 was found to be overexpressed in esophageal squamous cell carcinoma tissues and its level was linked to lymph node metastasis and tumor stage \(^{64}\).

Baek et al. \(^{61}\) and Huan et al. \(^{64}\) reported that in gastric and esophageal malignancies, MRP1 expression was associated with an advanced tumor stage, poor prognosis, and metastasis. They explained increased invasiveness of tumor cells resulting from the activation of signaling molecules, such as Phosphatidylidyinositol 4-kinase (PI4K) by the transcription of matrix metalloproteinase 2 caused by MRP1 crosslinking.

Another study by Kim et al. \(^{65}\) stated that MRP1 was found to be positively correlated with metastasis of lymph node. Epidermal growth factor receptor (EGFR) and discoidin domain receptor (DDR1) were associated with the MRPI which regulate cell motility. As a result of interaction with EGFR, tumor cells are able to extra- or intravasate by EGF-dependent chemotactic migration to extracellular matrix.

Baek et al. \(^{61}\) stated that MRP1 overexpression accelerated bone metastasis in human breast cancer. Another two studies found a metastasis enhancing role of MRP1 in gastric carcinoma. In the first report, MRP1 expression was overexpressed in primary and metastatic gastric cancer tissues than it was in the patient's adjacent stromal non-cancerous areas. They found also a strong association of elevated MRP1 expression levels with vessel invasion \(^{66}\). In the second one, MRP1 was strongly positivity linked with lymph node metastasis and venous invasion \(^{67}\).

3.3 Matrix Metalloproteinase-9 (MMP-9) in cancer metastasis

During tumor development, MMP-9 has a role in BM destruction that in turn helps in tumor invasion and metastases \(^{68}\). Matrix Metalloprotease-9 overproduction causes the primary ECM and BM constituents to degrade, allowing tumor cells to escape promoting metastasis. Studies by Pego et al. \(^{69}\) and Candido et al. \(^{70}\) described MMP-9 as a tumor microenvironment motivator as it aids tumor invasion, metastasis, and angiogenesis, in addition to ECM remodeling.

Several matrix metalloproteinas have been found to be overexpressed in advanced mammary tumors, and this contributes to tumor invasion, metastasis, and a worse outcome for breast cancer \(^{71}\). In numerous studies, MMP-9 high level has been found as an indicator for invasive ductal carcinoma and has been linked to tumor level \(^{72}\). Matrix Metalloprotease-9 was found in breast cancer cells to trigger signaling pathways that activate genes related to migration and invasion \(^{73},\ 74\). In a similar study by Li et al. on breast cancer tissues, they found
a correlation between MMP-9 expression level, lymph node metastasis and tumor stage.  

Furthermore, Gobin et al. found MMP-9 significantly overexpressed in RCC metastasis and angiogenesis. Li et al. stated that silencing of the MMP-9 expression boosted by transforming growth factor β1 (TGF-β1) blocks EMT and inhibits migration and metastasis of thyroid cancer cells as well as esophageal squamous cell carcinoma (ESCC) cells. Jiang and Li reported that in patients with brain cancer, MMP overexpression was linked to lymph node metastasis, proposing it to be used as an indicator for brain cancer metastasis. According to reports, MMP-9 is crucial for the invasion and metastasis of thyroid cancer cells as well as esophageal squamous cell carcinoma (ESCC).  

3.4 Vimentin in cancer metastasis  

Vimentin regulates cell invasion dependent on its unique structural and signaling characteristics. During cancer progression, vimentin promote metastasis via inhibition of focal adhesion-associated proteins. Vimentin controls endothelial cells' "cuplike" shape to promote cancer metastasis. Vimentin has been shown to be necessary for in vitro cancer-associated fibroblasts' motility during collective migration. Several investigations have also demonstrated vimentin's ability to promote EMT.  

Numerous studies have illuminated that vimentin level was strongly related to the emergence of tumor. Dauphin et al. stated that in 295 non-small cell lung cancer (NSCLC) patients, 145 had a vimentin-positive expression according to immunohistochemical detection results. Vimentin is primarily expressed in tissues that have undergone extensive metastatic spread. Meanwhile, the results of 193 patients' follow-up investigations revealed that expression of vimentin was strongly connected with NSCLC metastasis.  

Du et al. reported that vimentin overexpression indicated a lower chance of overall survival, a lower chance of being free from cancer, and more significant lymph node metastases, according to a study on 1,969 CRC patients. Furthermore, vimentin's significance in the lymphatic invasion and lymph node metastases of gastric, esophageal, and oral squamous cell carcinoma (OSCC) has been described in various instances. It has been demonstrated that OSCC lymph node metastasis was highly correlated with abnormal vimentin expression level. Bhardwaj et al. found that in OSCC originating from the sebaceous gland, elevated expression of vimentin was likewise substantially linked with metastasis of lymph node.  

Bu and chen reported that in OSCC-derived cell line Tca8113, metastasis and invasion of the OSCC were linked to the transforming growth factor 1 (TGF1)-dependent overexpression of vimentin. Blocking TGF1 reduced the capacity of these cells to migrate, pointing to vimentin's role in promoting TGF1-induced EMT.  

Moreover, Liu et al. have considered vimentin as the most optimistic marker among the five indicators concerning EMT vimentin, snail, Twist, N-cadherin and E-cadherin.  

3.5 Transforming growth factor β in cancer metastasis  

Many cancers have aberrant TGF-β signaling expression, including hepatocellular carcinoma, gastric, lung, and breast tumors. Transforming growth factor promotes tumor formation by promoting EMT, invasion and metastasis.  

According to reports by Im et al., TGF-β increased gastric cancer metastasis through upregulating MMP-9. Furthermore, Liu et al. stated that TGF-β was able to accelerate CRC growth and migration by inducing the EMT. Another study on CRCs by Zhao et al. stated that greater metastasis and invasiveness were associated with decreased E-cadherin expression, and activated TGF-β Smad signalling.  

Furthermore, TGF-β increased the contact between pericyte-endothelium and fibronectin, which in turn enhanced metastatic invasion of breast tumor. Transforming growth factor β also boosted breast cancer proliferation by MMP-9 dependent mechanism. Moreover, it stimulates urokinase plasminogen activator, which is important for the in vivo spread of aggressive breast cancer.  

Ashrafizadeh et al. stated that signaling of transforming growth factor β enables bone metastasis of prostatic cancer cells, in this case it was regarded to be a diagnostic and prognostic biomarker. Its signaling contributes to metastasis of prostatic cancer cells via EMT mechanism. This was supported by another study by Lee et al. who stated that TGF-1 exposure in human prostatic cancer cells caused EMT, enhanced invasiveness, and boosted metastasis.  

Transforming growth factor beta boosts macrophages to produce vascular endothelial growth factor (VEGF), and this was thought to have promoted tumor vascularization. According to Sun et al., TGF activated EMT like transition in human melanoma cells via platelet-derived growth factor (PDGF) and phosphatidylinositol 3-kinase (PI3K) signalling.
In another study by Bi et al., TGF-/Smad signaling was enhanced, facilitating HCC progression, migration and metastasis. Khatami stated that TGF promotes keratinocyte proliferation as well as EMT in pancreatic cells, and increases transcription of NF-xB target genes. Furthermore, Steinbichler et al. reported that pancreatic cancer cells’ exosomes motivated kupffer cells to produce TGF-β, fostering an inflammatory milieu that enhanced the pancreatic carcinoma metastasis.

Johansson et al. found that breast cancer cells acquired EMT characteristics and became sensitive to the TGF-β growth stimulatory effects, promoting their metastasis to the lungs of tumor-bearing mice.

3.6 Snail-1 in metastatic studies

Snail-1 is a vital transcription factor to EMT. It acts by suppressing E-cadherin, which causes N-cadherin protein and vimentin expression to be upregulated enabling tumor invasion. Cancer cell metastasis could be prevented by inhibiting Snail-1 through hindering EMT and invasiveness. Snail-1 was found in various cancer types, such as cervical, bladder, breast, hepatocellular and pancreatic carcinoma.

Hojo et al. found that the knockdown of Snail-1 expression in an animal model suppressed the metastasis of ovarian tumors via the upregulation of MMP expression.

Kuncman et al. demonstrated a substantial correlation between lymph node metastases and elevated expression levels of Snail-1, and TGF-1 in thyroid cancer patients’ specimens. Moreover, Wieczorek-Szukala et al. reported that there was a strong correlation between Snail-1 expression and the staging system of thyroid cancer. Mato et al. were successful in demonstrating that patient samples with thyroid cancer in stages I and II had over 8-fold greater levels of Snail-1 expression compared to controls.

Chen et al. recommended Snail-1 use in head and neck squamous cell carcinoma as a vital biomarker for lymph node metastasis. They clarified that by enhancing EMT process which favors tumor progression, in addition to the formation and collective migration of CTC clusters. They added that Snail-1 could promote angiogenesis by elevating VEGF expression. The primary controlling factor in tumor-induced lymphangiogenesis that encourages lymph node metastasis is vascular endothelial growth factor, which is released by malignant tumor cells. The VEGF boosts lymphatic vessel growth that surround the tumors to express the integrin 41, which couples with vascular cell adhesion molecule 1 to help lymph node metastasis and cancer cell migration.

Olmeda et al., Mahabir et al. and Hemmatzadeh et al. found that Snail-1 silencing downregulated vimentin, fibronectin and MMP-9 in human breast carcinoma, malignant glioma cell lines and esophageal Cancer Cells, respectively. They stated that EMT process promotes invasiveness and migratory properties of cells by differentiation of epithelial cell to become mesenchymal cells. In this process, common epithelial indicators including E-cadherin, Mucin-1, and cytokeratin are replaced by the expression of vimentin and fibronectin, which are mesenchymal markers. They further explained that protease enzymes play a crucial part in the EMT process, which promotes tumor cells’ ability to metastasize to other tissues through the extracellular matrix and its constituent parts. These enzymes participate in this process through destroying extracellular matrix constituents and dissolving the connections of connective tissues.

Snail-1 is supposed to be necessary for lymph node metastasis in human breast cancer. Additionally, it is believed that Snail-1 expression influences the ability of pancreatic tumor cells to metastasize. Therefore, Snail-1 may be a suitable candidate for targeting in order to stop metastasis.

3.7 Role of twinfilin in metastasis

Many studies have been done on the role of TWF in many diseases, including malignancies. Kaishang et al. stated that TWF could have a role in the metastasis of certain tumor types. Twinfilin has been found to promote cancer metastasis. Overexpression of Twinfilin boosts EMT process by turning on transcription factors that promote the mesenchymal lineage, like Mega karyoblastic leukemia 1(MKL1) and Serum response factor (SRF).

Kaishang et al. stated that in adenocarcinoma of the lung and squamous cell carcinoma patients, the expression of TWF, tumour growth and lymph node metastasis are in great correlation. Chen et al. found that increased TWF-induced aberrant EMT activation in lung adenocarcinoma causes tumor invasion, metastatic activation, the appearance of treatment resistance, and a poor prognosis. Twinfilin could alter mTOR activation to influence cell proliferation where lung cancer is linked to dysregulation of serine/threonine protein kinase mTOR.

Samaeekia et al. stated that in breast cancer, the level of miR206 was downregulated, then targets
twinfilin which leads to accelerated metastasis via IL11 signaling pathway. In another two studies on breast cancer conducted by Bockhorn et al. they found that the biomarker miR-30c for human breast tumor has a vital role in the prevention of metastasis by specifically targeting TWF, which activates EMT. In the other study, reduction of twinfilin in breast cancer cell line MDA-MB-231 cells have significantly led to less organised F-actin, decreased vinculin positive focal adhesions, and inhibited EMT and metastasis. Furthermore, Tian et al. reported that TWF overexpression promoted breast cancer cells EMT by the increase of cyclin D1 expression in breast epithelial cells.

Table (2): Summary of the diagnostic significance of the most important studied metastatic biomarkers

<table>
<thead>
<tr>
<th>Metastatic biomarker</th>
<th>Primary site</th>
<th>Secondary site</th>
<th>Marker level in metastasis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cadherin</td>
<td><em>lung adenocarcinoma</em></td>
<td><em>Lymph node or chest wall</em></td>
<td>Downregulation</td>
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<td></td>
<td><em>lung cancer</em></td>
<td><em>Brain</em></td>
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<td>51</td>
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<tr>
<td></td>
<td><em>Colorectal cancer</em></td>
<td><em>Lymph node</em></td>
<td></td>
<td>52</td>
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<tr>
<td>MRPI</td>
<td><em>Breast cancer</em></td>
<td><em>Bone</em></td>
<td>Upregulation</td>
<td>61</td>
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<tr>
<td></td>
<td><em>Gastric cancer</em></td>
<td><em>Lymph node</em></td>
<td></td>
<td>66</td>
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<tr>
<td></td>
<td><em>Esophageal squamous cell carcinoma</em></td>
<td><em>Lymph node</em></td>
<td></td>
<td>64</td>
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<td>MMP-9</td>
<td><em>Breast cancer</em></td>
<td><em>Lymph node</em></td>
<td>Upregulation</td>
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<tr>
<td></td>
<td><em>Brain cancer</em></td>
<td><em>Lymph node</em></td>
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<tr>
<td>Vimentin</td>
<td><em>CRC</em></td>
<td><em>Lymph node</em></td>
<td>Upregulation</td>
<td>85</td>
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<td></td>
<td><em>oral, gastric, esophageal and oral squamous cell carcinoma (OSCC)</em></td>
<td><em>Lymph node</em></td>
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<tr>
<td></td>
<td><em>OSCC</em></td>
<td><em>Lymph node</em></td>
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<td>TGF-β</td>
<td><em>Prostate cancer</em></td>
<td><em>Bone</em></td>
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<td></td>
<td><em>Breast cancer</em></td>
<td><em>Lung</em></td>
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<td>103</td>
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</table>

Table (3): Prognostic significance of studied metastatic biomarkers

3.1 Clinical outcome with low E-cadherin

<table>
<thead>
<tr>
<th>Type</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Pancreatic carcinoma</td>
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<td>HCC</td>
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<tr>
<td>OSCC</td>
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3.2 Clinical outcome with high MRPI

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>References</th>
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<tbody>
<tr>
<td>Papillary thyroid carcinoma</td>
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<tr>
<td>Gastric cancer</td>
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<tr>
<td>Cutaneous melanoma</td>
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<tr>
<td>Pancreatic carcinoma</td>
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</tr>
<tr>
<td>Lobular breast carcinoma</td>
<td>61</td>
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<tr>
<td>Breast cancer</td>
<td>129</td>
</tr>
<tr>
<td>Esophageal squamous cell carcinoma</td>
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<tr>
<td>Cervical cancer</td>
<td>131</td>
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<tr>
<td>Gallbladder cancer</td>
<td>132</td>
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<td>Breast cancer</td>
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3.3 Clinical outcome with high MMP9 level

<table>
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<tr>
<th>Type of cancer</th>
<th>References</th>
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</thead>
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<tr>
<td>Poor prognosis</td>
<td>Breast cancer 134</td>
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<tr>
<td>Poor prognosis</td>
<td>Gastric cancer 135</td>
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<tr>
<td>Poor prognosis</td>
<td>Colorectal cancer 136</td>
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<tr>
<td>Poor prognosis</td>
<td>Non small cell lung cancer 137</td>
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3.4 Clinical outcome with high vimentin level

<table>
<thead>
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<th>Type of cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor prognosis</td>
<td>Endometrial cancer 139</td>
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<tr>
<td>Poor prognosis</td>
<td>Colorectal cancer 140</td>
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<td>Poor prognosis</td>
<td>Lung adenocarcinoma 141</td>
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3.5 Clinical outcome with high TGF β level

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3.6 Clinical outcome with high Snail-1 level

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3.7 Clinical outcome with high Twinfilin 1 level

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4. CONCLUSIONS

In conclusion, this research highlights the significant potential of circulating biomarkers for the diagnosis and prognosis of cancer metastasis. The development of non-invasive and cost-effective diagnostic tools that can accurately detect and monitor cancer invasiveness is urgently needed in clinical practice. Circulating biomarkers offer a promising avenue for achieving this goal, providing valuable information on disease progression, treatment response, and patient outcomes. Reduced E-cadherin expression might be a good biomarker for predicting poor prognosis, Whereas MRP1, MMP9, vimentin, TGFβ, Snail-1 and Twinfilin low level is associated with good prognosis. However, further research is needed to validate these biomarkers and to develop standardized assays for their detection and quantification. With continued advancements in this field, we hope to see the translation of circulating biomarkers into clinical practice in the near future, ultimately improving the prognosis and quality of life for cancer patients.

Funding: This article did not receive a specific grant from any funding agency in the public, commercial or not for specific sectors.

Acknowledgments: NA

Conflicts of Interest: The authors declare that they have no competing interest.

Ethical Statement: The study was performed in line with the ethical rules and policies set out by the Ethics committee of faculty of pharmacy,(Girls), Al-Azhar university (No 151).

Author Contribution: This work was carried out in collaboration between all authors. Sara A. Gaballah: Investigation, Formal analysis, Resources and Writing Original Draft. Ahmed I. Eldesouky: Validation, Software, Editing manuscript, Supervision, Management and Coordination. Noha A. Eldesoky: Conceptualization, Visualization, Writing Review, Editing, Supervision and gaining ethical approval.


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